~~Description~~

SUBSTITUTE SPECIFICATION

METHOD FOR MEASURING THE CONCENTRATION OR CHANGE IN CONCENTRATION OF A REDOX-ACTIVE SUBSTANCE AND ASSOCIATED DEVICE

[0001] This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/EP2005/050333 which has an International filing date of January 26, 2005, which designated the United States of America and which claims priority on German Patent Application number 10 2004 004 654.9 filed January 29, 2004, the entire contents of which are hereby incorporated herein by reference.

Field

[0002] The invention generally relates to an electrochemical measuring method for measuring the concentration or change in concentration of a redox-active substance. In addition, the invention also generally relates to an associated device, such as one for carrying out the measuring method for example, with an electrochemical transducer array.

Background

[0003] Amperometric sensors are based on the simple principle that reducible or oxidizable substances can be converted by applying a corresponding potential to an electrode. The so-called Faraday current that then flows is a measure of the concentration of this substance. This current cannot, however, be measured directly after the potential has been applied, because above all a high capacitive current flows in the first instance. This is brought about by the charge-reversal

of the double layer of the electrode. It dies down exponentially with time.

[0004] The Faraday current is likewise reduced with time, since as a result of the substance-conversion, a depletion takes place in front of the electrode. The subsequent delivery of substance from the solution to the electrode takes place solely as a result of diffusion in the case of non-stirred, that is, non-convective systems. Concentration profiles set in in the solution. With sufficiently positive potentials (oxidation) or negative potentials (reduction), the Cotrell equation holds good for the time-dependence of the current:

$$ I = nFA \ \frac{\sqrt{D}}{\sqrt{\pi t}} \cdot c_\infty \ , \ s \ -> \infty \tag{1} $$

[0005] The significations in this connection are:

n: number of electrons per formula conversion
F: Faraday constant
A: area of the electrode
D: diffusion coefficient
c_∞:   concentration of the substance to be converted in the solution
s: extent of the electrolyte space in front of the electrode

[0006] Equation (1) implies that the Faraday current diminishes with the reciprocal square root of time, that is, more slowly than the capacitive current. Some time after the change in the potential, the current is therefore mainly determined by the Faraday current. For each system it is necessary to find a point in time at which the capacitive current is low, yet the Faraday current is still as high as possible.

[0007] One possibility for determining very small concentrations (10^{-10} mol/l) is voltammetric stripping analysis. In this connection, by applying a suitable potential the substance that is to be determined is first enriched by that of the electrode. In a second step, the quantity of substance on the electrode is then determined [W. Buchberger, Elektrochemische Analyseverfahren, Heidelberg Berlin: published by Spektrum Akademischer Verlag in 1998 and DE 44 24 355]. This method does not enable there to be any time resolution on account of the two method steps. Changes in concentration cannot therefore be tracked or can only be tracked very slowly.

[0008] A further analytical standard method for determining very small concentrations is polarography [Rach & Seiler, Polarographie u. Voltametrie in der Spurenanalytik, Heidelberg: Hüthig 1984]. Here concentrations in the order of magnitude of 10^{-9} mol/l can be measured with suitable pulse methods and with the use of a so-called "Dropping Mercury Electrode" (DME). In the case of the DME, use is made *inter alia* of the fact that each drop of mercury plunges into the solution anew and the concentration c_∞ is found there again. In the case of a static electrode, however, this is not the case. After each measurement it would be necessary to wait for so long until the concentration profile is relaxed by the diffusion process. Depending on the magnitude of the diffusion coefficient, if there is no stirring this process can take a few seconds. If the waiting time is not long enough, the current will diminish from measurement to measurement, since the initial concentration drops in front of the electrode.

[0009] In the case of some applications, however, it can be necessary to determine the concentration in

rapid succession, if, for example, a change in
concentration is to be observed. One possible area of
use is that of tracking the enzymatic formation of a
mediator in molecular-biological detection systems.

[0010] In the case of biochemical sensors, molecular
recognition systems, for example haptens, antigens or
antibodies, are placed on or in the vicinity of the
electrodes. The target molecule binds thereto and is
provided with an enzyme label either directly or by way
of intermediate steps. If the corresponding enzyme
substance is now added, the enzyme releases a substance
that can be detected. This happens either visually or
electrochemically. This is the so-called ELISA-test
(Enzyme Linked Immuno Sorbent Assay). DNA analysis
methods can also be carried out in a similar way.

[0011] In the case of electrochemical detection, it is
advantageous to detect not just the absolute
concentration of the measured variable, referred to as
a "mediator", for the electrochemical conversions
specified above, but also the change, in particular the
increase, in the concentration over a few seconds. As
a result, the influence of different states of the
biochemical system is eliminated at the start of the
measurement. The time resolution of such a measurement
must then amount to 1 to 2 Hz in order to be useful
technically and economically.

[0012] Specifically in the case of redox-active
substances up until now it has been possible to employ
so-called redox-cycling with the use of interdigital
electrodes. In so doing, use is made of the fact that
the substance that is oxidized at one electrode can be
reduced again at the second electrode. The electrodes
are then constantly set to the oxidation or reduction
potential. To this end, the two interdigital

electrodes with electrode fingers interlocking in a ~~chamber~~comb-like manner are connected together with the reference electrode and a counter electrode to a bipotentiostat [O. Niwa, M. Morita, H. Tabei, Anal. Chem. 62 (1991), 447-452 and DE 43 18 519 A1].

[0013] A precondition for redox-cycling is that the distance between the electrodes, that is, the electrode fingers of the interdigital electrodes that are associated with each other, lies in the order of magnitude of the diffusion-layer thickness, that is, in the region of a few μm. On the basis of the concentration profiles, in addition to the concentration and the diffusion coefficient the number of electrode fingers and their length enter into the measured current [K. Aoki, J. Electroanal. Chem, 270 (1989), 35]. It follows from this that the necessary structures must be very fine and expensive to produce.

[0014] The monograph "Electrochemical Methods, Fundamentals and Applications", John Wiley & Sons, 1980, provides a general overview of electrochemical measuring technology. Further references to measurement specifically with respect to liquids or even for biochemical measurements are given in DE 43 35 241 A1, in DE 41 31 731 A1, in DE 197 17 809 U1 and DE 199 17 052 A1. A method for electrochemically measuring redox-cycling with a practice-related electrode arrangement is described in detail in WO 01/67587 A1.

SUMMARY

[0015] ~~Basing considerations on the latter prior art,~~ ~~i~~Int ~~is an object of~~ at least one embodiment of the invention, ~~to put forward~~ a method is put forward in which the determination of concentrations of a redox-active substance in μm-concentrations is possible on

areal electrodes with diameters of $\geq$ 30 µm, preferably
$\geq$ 50 µm. In this connection, the system is not to be
convective, that is, neither the electrode nor the
solution is to be stirred or moved, and the measuring
frequency is to amount to $\geq$ 1Hz. In addition, an
associated measuring device is to be provided.

[0016] The object is achieved in accordance with the
invention in the case of a method of the kind mentioned
at the beginning by means of the measures of claim 1.
An associated measuring device is specified in claim
11. Further developments of the method in accordance
with the invention and the associated measuring device
are subject matter of the dependent claims.

[0016] It is proposed that the potential of the working
electrode be pulsed in a manner known per se. In the
case of at least one embodiment of the invention,
however, measuring phases and also relaxation phases
are formed alternately, with the measuring-phase pulse
lengths being selected so that towards the end of the
pulse the capacitive current is small in comparison
with the Faraday current, and with the relaxation-phase
pulse lengths being selected so that towards the end of
the pulse the concentration gradient is relaxed so that
at the beginning of the following measuring phase the
change in concentration of the mediator, brought about
by the consumption of the mediator by the measurement
itself, is reversed to the greatest possible extent.
Thus, the current that is measured at the end of the
measuring phase forms a significant measuring signal.

[0017] In accordance with at least one embodiment of
the invention, rapid measurement of the concentration
and in particular of the change in concentration of a
mediator in molecular-biological detection systems by
meansway of the electrochemical redox-reaction of the

enzymatically formed redox-active substance, where the
working electrodes are anchored in caverns on a chip
band, is effected by cyclic pulse-loading, with the
current that is measured after the charge-reversal of
the double layer has died down forming the measuring
signal.

[0018] ~~The~~ A basis of <u>at least one embodiment of</u> the
invention is the recognition that by using a new redox-
cycling variant advantageously it is possible to carry
out measurements at electrodes with diameters in the
order of magnitude of a few 100 μm up to 1 cm.
Complexly constructed interdigital electrodes are no
longer necessary. Now inexpensive transducer arrays
can be used, as described in detail in particular in
Patent Application Serial Number 10 2004 004 654.9-52
belonging to the applicant and having the same filing
priority<u>, the entire contents of which is hereby
incorporated herein by reference</u>.

[0019] In the case of the associated measuring device,
the measuring installation is simplified with respect
to redox-cycling to the effect that no bipotentiostat
is required. One single potentiostat in combination
with a pulse generator suffices.

[0020] In the case of <u>at least one embodiment of</u> the
invention, it is not a stationary state - as in the
case of "normal" redox-cycling - that is set, but
instead a rapid relaxation of the concentration
gradient that is forced electrochemically. To this
end, the potential of the working electrode is pulsed.
A diffusion layer is formed whose thickness at the end
of the measuring period attains a maximum value that is
dependent upon the length of the measuring phase.

[0021] When measuring oxidation currents, an adequate reduction potential is set during the relaxation phase. The species oxidized during the measuring phase and still located in front of the electrode are therefore reduced again. The concentration gradient and thus the diffusion layer are broken down. Instead of therefore, as in the case of redox-cycling, establishing constant diffusion-layer thicknesses, in the case of pulsed redox-cycling in accordance with the invention the diffusion layer is built up and broken down again with time.

[0022] In both cases, as a result a diffusion-layer thickness is set that is limited at least in terms of its maximum value. If a reduction is to be observed, the reduction potential must be set during the measuring phase and the corresponding oxidation potential must be set during the relaxation phase.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Further details and advantages of the invention emerge from the following description, with reference to the figures, of ~~exemplary~~ example embodiments in connection with the claims and with the aid of the drawings, in which:

Figure 1   shows a graphic representation of pulsed redox-cycling with characteristic lines for pulse lengths and associated concentration and signal curves;

Figure 2   shows a graphic representation of the dependence of the relative concentration upon the spacing of the electrodes;

Figure 3   shows a graphic representation of the current as a function of the concentration and

Figure 4   shows a graphic representation of the rise as
            a function of the potential during the
            relaxation phase;

Figure 5   shows a graphic representation of the
            dependence of the current density upon time;

Figure 6   shows a representation in accordance with
            Figure 5 for the relaxation phase;

Figure 7   shows a representation in accordance with
            Figure 5 with comparison of experimental and
            calculated values;

Figure 8   shows a graphic representation of the
            dependence of the concentration upon the
            electrode spacing;

Figure 9   shows a measuring device for pulsed redox-
            cycling with an associated transducer array;

Figures 10/11 show the transducer array for the
            measuring device in accordance with Figure 9
            in the view from above and below; and

Figure 12 shows the course of a typical measurement
            with a measuring-current curve.

DETAILED DESCRIPTION OF THE EXAMPLE EMBODIMENTS

[0024] The methodology of pulsed redox-cycling is
described in the first instance in Figures 1 to 4.   In
this connection, it is assumed that the methodology of
redox-cycling is known per se from the prior art.   Then
with reference to Figures 5 to 8 observations are made
with regard to the accuracy, and finally in Figure 9 a
measuring device with an ~~exemplary~~ example transducer

array, whose structure is shown in Figures 10 and 11, is specified. Finally, Figure 12 depicts the direction of the method when the arrangement is used as a DNA sensor.

[0025] The different phases during measurement are characterised in Figure 1. Which is the measuring phase and which is the relaxation phase depends upon whether an oxidation current or a reduction current is to be measured. The pulse lengths for oxidation and reduction need not be the same, that is, the times Δt_{ox} and Δt_{red} can differ. In addition, the potentials φ_{ox} and φ_{red} need not be symmetrical with respect to the redox-potential φ_0 of the species.

[0026] The representation of Figure 1 shows the possible course of the potential with the use of rectangular pulses. In this connection, the potentials φ_{ox} φ_{red} and the times Δt_{ox} and Δt_{red} are plotted in arbitrary units with the pulse shape 11. The pulse shape 11 with the pulse lengths for oxidation and reduction does not need to be symmetrical. Pulsed redox-cycling can be realized in exactly the same way with delta voltage curves or sine curves.

[0027] In particular, in Figure 1 the measuring phase is denoted by A and the relaxation phase is denoted by B. Besides the potential curve 11, a curve 12 for the concentration of the mediator of the electrode and in addition to this the electric current 13 as a measuring signal are further represented. The current value that is relevant for the process is therefore present at the respective end of the relaxation interval and is a measure of the relaxed change in concentration.

[0028] In the case of the further observations that are made it is assumed that the concentration of a molecule

that is present in its reduced form is to be measured.
In this case, during the measuring phase the potential
is related in a positive way to the redox potential of
the species. The highest measurement currents are
achieved if the potential lies so far in the positive
area that a diffusion limiting current sets in. The
current is then not limited by the kinetics of the
redox reaction, but just by the diffusion. The
development of the concentration profile of the reduced
species over time follows from the corresponding
solution of Fick's laws of diffusion in the simple
infinite half-space.

$$c(t,x) = c_\infty \cdot \mathrm{erf}\left(\frac{x}{2\sqrt{Dt}}\right), \quad s \rightarrow \infty \qquad (2)$$

[0029] The significations in this connection are:

c: concentration as a function of time and place

c_∞: concentration in the solution ($x \rightarrow \infty$)

erf: error function

D: diffusion coefficient

s: extent of the electrolyte space in front of the
electrode

[0030] Two concentration profiles 21 and 22 for a
substance with the diffusion coefficient D = 3.6 E-6
cm²/s are shown in Figure 2. This corresponds to the
diffusion coefficient of para-aminophenol (pAP), by way
of the example of which the mode of operation of pulsed
redox-cycling shall be demonstrated below.
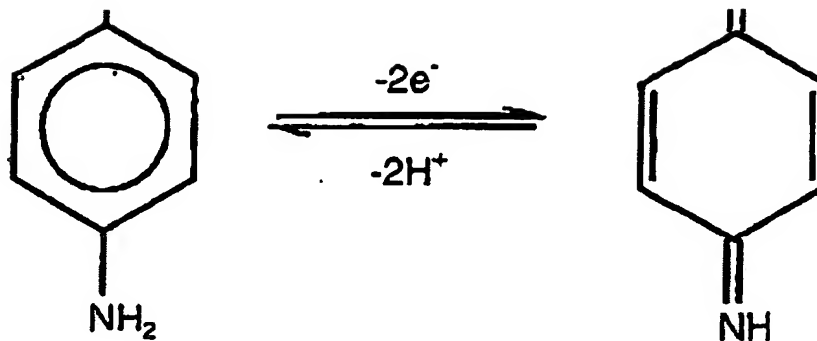
para-aminophenol   quinonimine

[0031] After 0.1 s, the diffusion layer has a thickness of approximately 25 μm. After 0.25 s, the extent of the layer depleted with respect to pAP already amounts to 40 μm. The thicker this layer is, the longer the relaxation lasts as a result of diffusion.

[0032] Figure 3 shows the current density as a function of the pAP concentration in the solution for various pulse trains that are characterised by characteristic lines 31 to 34. The measuring phase always amounts to 0.25 s, the relaxation phase to 0.75 s. The measurement of current takes place 0.24 s after the start of the measuring phase. The oxidation potential during the measuring phase relative to the redox potential amounts to + 200 mV. The potential has been varied during the relaxation phase. It assumes values between -300 mV and 0 mV relative to the redox potential. In particular:

$\varphi_{red}/\varphi_{ox}$ is -300 mV/200 mV for characteristic line 31
        -200 mV/200 mV for characteristic line 32
        -100 mV/200 mV for characteristic line 33
and   0 mV/200 mV for characteristic line 34.
Given these parameters, different rises in the current density 5 in terms of its functional dependence upon the concentration pAP result.

[0033] The rise in the current density with the pAP-concentration, that is, the sensitivity of the measurement, increases constantly with a potential that is becoming negative during the relaxation phase.

Plotting the rise against the relaxation potential in accordance with Figure 4 clearly shows, by ~~means~~way of characteristic line 41, the advantageous effect of pulsed redox-cycling.

[0034] Whilst in the case of a relaxation potential of 0 V versus φ_0 the rise only amounts to 462 Acm/mol, this value increases to 864 Acm/mol at -300 mV versus φ_0. This doubling is due to the improved effect of the redox-cycling, because given a relaxation potential of -300 mV versus φ_0 the quinonimine in front of the electrode is completely reduced to para-aminophenol (pAP). Given a relaxation potential of 0 V versus φ_0, on the other hand, in accordance with the Nernst equation directly in front of the electrode a concentration ratio of para-aminophenol:quinonimime = 1:1 is set. The quinonimine is thus only partly further reduced.

[0035] The rise in sensitivity is only one advantage of pulsed redox-cycling. The constancy of the current signal already during the first seconds of measurement is a further important advantage.

[0036] The current density that has set in after a comparatively long measuring time was used to determine the sensitivity. When measuring enzyme activities, for example, the measurement is, however, effected in such a way that the solution is first stirred further or pumped. The pAP formed by the enzyme is rinsed away as a result and a constant basic current sets in. Then the pump is stopped and the rising concentration is measured during the first seconds. Typical rises then lie in the order of magnitude of 2 $\mu A/cm^2 s$. If the measurement itself now results in a drop in the signal, both effects are superimposed and too small a rise in the current and thus in the enzyme activity is

measured. Since this drop in the current as a result
of the consumption of the substance depends, moreover,
upon the concentration thereof, this effect cannot be
eliminated by standardization.

[0037] Experiments with constant concentration provide
information about the signal constancy over time. The
concentration amounted to 50 µM pAP, the potential
during the measuring phase to +200 mV. The duration of
the measuring phase amounted to 250 ms, in which case
the measurement of current took place after 240 ms.
The potential during the relaxation phase amounted in
the first experiment to 0V versus φ_0, in the second
experiment to -300 mV versus φ_0. The duration of the
relaxation phase was varied between 250 ms and 4.75 s.

[0038] The dependence of the current density j upon
time is reproduced in Figure 5 in a graphic
representation. Characteristic lines 51 to 54 result
for different relaxation-phase durations Δt_{red}, that is,
in particular between 0.255 and 4.755. The currents
fall greatly within the first 10 s of measurement.
Given a length of the relaxation phase of 0.25 s, the
decrease amounts to 14 $\mu A/cm^2$ in 10 s. If the duration
of the relaxation phase is increased to 4.75 s, the
decrease in the signal reduces to 9 $\mu A/cm^2$ in 10 s.
The shorter the duration of the relaxation phase is
therefore, the greater this decrease in the signal is
with time. However, even with long relaxation times,
the decrease of 0.9 $\mu A/cm^2$ is still considerable
compared with the rises that are to be measured in the
application in the order of magnitude of 2 $\mu A/cm^2s$.

[0039] If the potential is now reduced during the
relaxation phase to -300 mV, the signal constancy is
significantly improved. This follows in particular
from Figure 6 which shows a representation that

corresponds to Figure 5 with characteristic lines 61 to
64 for the same parameters of the relaxation phases
Δt_{red}.

[0040] Given a relaxation time of 0.25 s, the signal
drop still amounts to 8 $\mu A/cm^2$ in 10 s. With a
relaxation time of 0.75 s, this value still amounts to
2 $\mu A/cm^2$ in 10s, with 1.75 still 1 $\mu A/cm^2$ in 10 s and
for 4.75 s only 0.5 $\mu A/cm^2$ in 10 s. Already with a
relaxation time of 0.75 s, that is, a measuring
frequency of 1 Hz, and a relaxation potential of -300
mV, the signal drop and thus the error as well only
amount to approximately 1 % of the expected measured
value.

[0041] The experiments show the influence of the
duration and potential of the relaxation phase on the
measuring signal. Simulation calculations can provide
further information on the effect of pulsed redox-
cycling. In this connection, on the one hand, the
current density is calculated in the case of redox-
cycling and, on the other hand, the current density
without redox-cycling is determined for comparison
purposes.

[0042] For the simulations with redox-cycling it was
assumed that the electrolyte space has a thickness of
100 μm. Both the oxidation potential and the reduction
potential are selected so that the reaction occurs in
the diffusion limiting current range, that is, the
currents are at a maximum. The pulse lengths are 250
ms at the oxidation potential and 750 ms at the
reduction potential. In the calculations without
redox-cycling, the parameters were the same apart from
the fact that during the relaxation phase no potential
is predetermined and no current can flow by way of the

potentiostat. During this time, the system is therefore decoupled electrochemically.

[0043] The comparison of the simulation data is effected with the experimental results for the corresponding pulse lengths and the potentials φ_{ox} = +200 mV and φ_{red} = -300 mV. These potential limits correspond best of all to the stipulations for the simulation. The y-axis sections of the simulation data were adapted to the experimental results.

[0044] Figure 7 shows good correspondence between the experiment and simulation for pulsed redox-cycling, with 71 representing the measured values and 72 representing the calculated characteristic lines. The drop in the current density is small under these conditions with 2 $\mu A/cm^2$ in 4 s. Without redox-cycling, on the other hand, the drop in current density during the first 4 s already amounts to 12 $\mu A/cm^2$, this being illustrated by the characteristic line 73. The results for pulsed redox-cycling with potentiostat-operation during the relaxation phase at redox potential lie in between, this being reproduced by characteristic line 74.

[0045] The improvement in the signal constancy by the factor 6 can be directly attributed to the concentration profiles. The following representation shows the calculated concentration profiles of pAP as a function of the spacing of the electrode, as they appear at the end of the 5th relaxation phase.

[0046] In the case of pulsed redox-cycling, during the relaxation phase, the previously formed oxidation product is reduced again. Consequently, the concentration of pAP directly in front of the electrode at the end of the relaxation phase has risen back to

the original value c_∞. Further away from the electrode, the concentration is only slightly diminished. Without redox-cycling, on the other hand, the concentration in front of the electrode immediately before the next measuring phase only amounts to 38 %. Also further away from the electrode, the concentration is clearly lower.

[0047] This follows in particular from Figure 8 with characteristic lines 81 and 82. In this connection, the example, in accordance with characteristic line 82 for measurement without redox-cycling, in practice corresponds to the measurement of the concentration of a substance which can admittedly be oxidized, yet whose oxidation products cannot be reduced again. The case would also be similar for a substance that reduces, but whose reduction products cannot be oxidized again. In the case of a biochemical sensor, this could, for example, be naphthol, which like pAP as well can be released during an enzymatic reaction.

[0048] The measuring device can be seen in detail in Figure 9. Apart from being realized by ~~means of~~ a transducer array 100 which is described further in detail with the aid of Figures 10 and 11, the measuring device is substantially realized by ~~means of~~ a suitable potentiostat 5 in combination with a pulse generator 6 which delivers optionally rectangular, triangular or sinusoidal pulses. By ~~means~~use of two operational amplifiers 7 and 7', of which one is connected to ground potential, and a defined measuring resistor 8, the potentiostat 5 is conceived in such a way that suitable potentials are provided. In this connection, the pulse length, the repetition rate and the level of the potential can be ~~predetermined~~set. In particular, the pulse lengths of the measuring phases and the relaxation phases can be set separately and be of

different lengths. The potentials can also be of
different magnitudes.

[0049] Associated with the transducer array 100 there
are individual electrodes 4a-4c which, in accordance
with the intended purpose, realize a reference
electrode REF, a counter electrode CE and at least one
measuring or working electrode WE. These electrodes
are connected to the potentiostat 5 as a three-
electrode arrangement. The signal of the potentiostat
5 is linked up to a signal-processing unit, which is
not shown in detail in Figure 9, with which evaluation
takes place in consideration of the above statements
regarding measuring methodology and accuracy.
Generally, the signal pattern shown in Figure 9 as I_{out}
results for the evaluation.

[0050] The transducer array 100 is reproduced in
Figures 10/11 as part of the measuring device that is
planar and flexible and in particular can be produced
inexpensively as well. What is important in this
connection is that now it is possible to carry out
measurements for pulsed redox-cycling with one
simplified transducer array 100. Figures 10 and 11
show the upper and lower side of the transducer array
100 - consisting of a metal substrate 1 and an
insulator layer 2. Circular depressions 3_i that are
referred to as cavities are shown on the upper side,
for example. The cavities 3_i develop as a result of the
structuring of the insulator 2. The upper side of the
metal substrate lies open at the base of the
depressions 3_i and forms a measuring point, if an
analytical unit is applied.

[0051] The representation of the rear side shows by
~~means~~way of lines a structuring and thus a sectioning
of the metal substrate 1 into portions that are

isolated from each other. Each metal island corresponds with a depression 31 [sic] 3i on the front side. The possible contact points for a so-called needle card for simplified electrical contacting of the metal surfaces are indicated by dots. What is important in this connection is that a plurality of metal islands, preferably three, with an analytical unit define a sensor, and with the associated electrodes 15, that is, a measuring electrode WE, a counter electrode GECE [sic] and a reference electrode REF, are suitable for carrying out electrochemical measurements.

[0052] Further specific electrode arrangements are described in a parallel application with the title "Elektrochemisches Transducer-Array und dessen Verwendung", belonging to the applicant and having the same filing priority.

[0053] A biochemical sensor for DNA analysis is realized with an arrangement in accordance with Figure 9 and a transducer array 100 in accordance with Figures 10/11, for example. The transducer array 100 that is described with the aid of Figures 10/11 and consists of a metal layer and an insulator layer that is connected therewith and has cavities 3i3i is used. The diameter of the cavities 3i3i amounts to 0.8 m, the depth to 90 μm and the spacing between two adjacent electrodes to 1 mm. The electrode surfaces are covered with a 2.3 μm thick gold layer.

[0054] All in all, in at least one example embodiment, the sensor arrangement consists of four electrodes for this intended application. One of the electrodes as a reference electrode is coated with a silver/silver chloride (Ag/AgCl)-layer, another electrode is used as

a counter electrode CE and the two further electrodes
serve as measuring electrodes WE.

[0055] On one of the measuring electrodes a synthetic
oligonucleotide sequence of length 25 is anchored by
~~means~~way of a terminal thiol group on the gold surface
as a positive test unit. The second measuring
electrode remains free as a negative test unit. Then
both surfaces are incubated with a solution of 1 mg
bovine serum albumin per ml for 15 minutes and
subsequently the sensor array is inserted into a 100 μm
deep through-flow channel. In the first instance 10 μl
of a 10 μM biotinylated target sequence is pumped over
the electrodes within approximately 5 minutes. Then
after a washing step, a solution of streptavidine of
marked alkaline phosphatase is passed thereover.

[0056] Washing is effected with a buffer solution of
100 mM tris(hydroxymethyl)aminomethane titrated to pH 8
with hydrochloric acid, 130 mM NaCl. After repeated
washing, a 2 mM solution of the enzyme substrate para-
aminophenylphosphate (pAPP) in the buffer solution is
pumped over the sensor array. In the presence of the
enzyme alkaline phosphatase, the enzyme substrate pAPP
is converted into para-aminophenol (pAP).

[0057] The reference electrode RE$\underline{F}$, counter electrode
CE and in each case one of the two measuring electrodes
WE are each connected to a potentiostat in a three-
electrode arrangement. The measurement is effected by
~~means~~way of pulsed redox-cycling. During the measuring
phase, the para-aminophenol formed by the enzyme is
oxidized to form quinonimine. The oxidation potential
φ_{ox} amounts to +200 mV versus $\underline{\varphi_o}\varphi^\theta$. In the relaxation
phase, the quinonimine that is formed is reduced back
to para-aminophenol at $\varphi_{red} = -200$ mV. The pulse length
of the measuring phase amounts to 250 ms, that of the

relaxation phase to 750 ms. The current is measured
240 ms after the start of the measuring phase.

[0058] At the beginning of the experiment, the positive
test unit, that is, the electrode with the catcher
sequence, is connected. The solution with the enzyme
substrate flows - conveyed by a pump - in the first
instance over the negative test unit and then over the
positive test unit. As a result of the flow movement,
pAP formed by the enzyme is rinsed away from the
electrodes so that when the pump is switched on the
current is constant and low. If the pump is now
stopped, the pAP concentration rises as a result of the
enzyme activity with time. In the measurement, this is
shown by a great rise in the current signal with 20
nA/s. If the pump is switched on again, the signal
drops back to the original value. This process can be
repeated as often as desired.

[0059] Figure 12 shows the course of the current over
time with the pump "on"/"stop" at the sensor
arrangement that has been described with a positive and
a negative test unit. The characteristic line 121
shows the course of the pump current. A specific
course with individual peaks ensues for the experiment,
with parameters the activation of the pump on the one
hand ("stop"/"on") and the switch-over of the measuring
electrodes, on the other hand. The measuring range of
interest is shown underneath in each case in broken
lines.

[0060] When t = 400 s, there has been a switch-over to
the negative test unit. Here the current drops in the
first instance when the pump is stopped, then remains
constant for a short time, and then slowly rises. This
rise is brought about by the diffusion of pAP from the
positive to the negative test unit. With the pump

"on", a peak current appears, since the electrolyte first flows from the positive to the negative test unit and thus an increased pAP-concentration is transported to the adjacent electrode. All in all, the discrimination between the positive and negative test unit is very good.

[0061] In an alternative measuring arrangement of at least one embodiment, instead of the self-supporting and flexible transducer array in accordance with Figures 9 to 11, a transducer array with the use of thin-film technology on a rigid substrate is employed. In this connection, areal electrodes are provided that have an extent that is greater than the length of diffusion. A typical diffusion length for the example specified above amounts to 25 µm so that the areal electrodes have an extent $\geq$ 30 µm, preferably $\geq$ 50 µm.

[0062] The rigid substrate is in particular silicon, which is preferably provided with an insulator layer.

[0063] It is also possible to carry out the redox-cycling in accordance with at least one embodiment of the invention with such transducers that enable CMOS technology to be accessed for signal-processing.

[0064] Example embodiments being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.